

MOUSE *Fkh<sup>f</sup>* cDNA SEQUENCE

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1  GCTGATCCCC CTCTAGCAGT CCACTTCACC AAGGTGAGCG AGTGTCCCTG
51  CTCTCCCCCA CCAGACACAG CTCTGCTGGC GAAAGTGCCA GAGAGGTATT
101 GAGGGTGGGT GTCAGGAGCC CACCAGTACA GCTGGAAACA CCCAGCCACT
151 CCAGCTCCCG GCAACTTCTC CTGACTCTGC CTTCAGACGA GACTTGGAAG
201 ACAGTCACAT CTCAGCAGCT CCTCTGCCGT TATCCAGCCT GCCTCTGACA
251 AGAACCCAAT GCCCAACCCT AGGCCAGCCA AGCCTATGGC TCCTTCCTTG
301 GCCCTTGGCC CATCCCCAGG AGTCTTGCCA AGCTGGAAGA CTGCACCCAA
351 GGGCTCAGAA CTTCTAGGGA CCAGGGGCTC TGGGGGACCC TTCCAAGGTC
401 GGGACCTGCG AAGTGGGGCC CACACCTCTT CTTCTTGAA CCCCCTGCCA
451 CCATCCCAGC TGCAGCTGCC TACAGTGCCC CTAGTCATGG TGGCACCCTC
501 TGGGGCCCCG CTAGGTCCCT CACCCACCT ACAGGCCCTT CTCCAGGACA
551 GACCACACTT CATGCATCAG CTCTCCACTG TGGATGCCCA TGCCAGACC
601 CCTGTGCTCC AAGTGCCTCC ACTGGACAAC CCAGCCATGA TCAGCCTCCC
651 ACCACCTTCT GCTGCCACTG GGGTCTTCTC CCTCAAGGCC CGGCCTGGCC
701 TGCCACCTGG GATCAATGTG GCCAGTCTGG AATGGGTGTC CAGGGAGCCA
751 GCTCTACTCT GCACCTTCCC ACGCTCGGGT ACACCCAGGA AAGACAGCAA
801 CCTTTTGGCT GCACCCCAAG GATCCTACCC ACTGCTGGCA AATGGAGTCT
851 GCAAGTGGCC TGGTTGTGAG AAGGTCTTCG AGGAGCCAGA AGAGTTTCTC
901 AAGCACTGCC AAGCAGATCA TCTCCTGGAT GAGAAAGGCA AGGCCAGTG
951 CCTCCTCCAG AGAGAAGTGG TGCAGTCTCT GGAGCAGCAG CTGGAGCTGG
1001 AAAAGGAGAA GCTGGGAGCT ATGCAGGCC ACCTGGCTGG GAAGATGGCG
1051 CTGGCCAAGG CTCCATCTGT GGCCTCAATG GACAAGAGCT CTTGCTGCAT
1101 CGTAGCCACC AGTACTCAGG GCAGTGTGCT CCCGGCCTGG TCTGCTCCTC
1151 GGGAGGCTCC AGACGGCGGC CTGTTTGAG TGCGGAGGCA CCTCTGGGGA
1201 AGCCATGGCA ATAGTTCCCT CCCAGAGTTC TTCCACAACA TGGACTACTT
1251 CAAGTACCAC AATATGCGAC CCCCTTTCAC CTATGCCACC CTTATCCGAT
1301 GGGCCATCCT GGAAGCCCCG GAGAGGCAGA GGACACTCAA TGAAATCTAC
1351 CATTGGTTTA CTCGCATGTT CGCCTACTTC AGAAACCACC CCGCCACCTG
1401 GAAGAATGCC ATCCGCCACA ACCTGAGCCT GCACAAGTGC TTTGTGCGAG
1451 TGGAGAGCGA GAAGGGAGCA GTGTGGACCG TAGATGAATT TGAGTTTCGC
1501 AAGAAGAGGA GCCAACGCCC CAACAAGTGC TCCAATCCCT GCCCTTGACC
1551 TCAAAACCAA GAAAAGGTGG GCGGGGGAGG GGGCCAAAAC CATGAGACTG
1601 AGGCTGTGGG GGCAAGGAGG CAAGTCCTAC GTGTACCTAT GGAAACCGGG
1651 CGATGATGTG CCTGCTATCA GGGCCTCTGC TCCCTATCTA GCTGCCCTCC
1701 TAGATCATAT CATCTGCCTT ACAGCTGAGA GGGGTGCCAA TCCCAGCCTA
1751 GCCCCTAGTT CCAACCTAGC CCCAAGATGA ACTTTCCAGT CAAAGAGCCC
1801 TCACAACCAG CTATACATAT CTGCCTTGGC CACTGCCAAG CAGAAAGATG
1851 ACAGACACCA TCCTAATATT TACTCAACCC AAACCCTAAA ACATGAAGAG
1901 CCTGCCTTGG TACATTCTGT AACTTTCAAA GTTAGTCATG CAGTCACACA
1951 TGACTGCAGT CCTACTGACT CACACCCCAA AGCACTCACC CACAACATCT
2001 GGAACCACGG GCACTATCAC ACATAGGTGT ATATACAGAC CCTTACACAG
2051 CAACAGCACT GGAACCTTCA CAATTACATC CCCCCAAACC ACACAGGCAT
2101 AACTGATCAT ACGCAGCCTC AAGCAATGCC CAAAATACAA GTCAGACACA
2151 GCTTGTGAGA

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Figure 1

## MOUSE Fkh<sup>+</sup> PROTEIN SEQUENCE

1	MPNPRPAKPM	APSLALGPSP	GVLPSWKTAP	KGSELLGTRG	SGGPFQGRDL
51	RSAHTSSSL	NPLPPSQLQL	PTVPLVMVAP	SGARLGPSPH	LQALLQDRPH
101	FMHQLSTVDA	HAQTPVLQVR	PLDNPAMISL	PPPSAATGVF	SLKARPGGLPP
151	GINVASLEWV	SREPALLCTF	PRSGTTPRKDS	NLLAAPQGSY	PLLANGVCKW
201	PGCEKVFEEP	EEFLKHCQAD	HLLDEKGKAQ	CLLQREVVS	LEQQLLEKE
251	KLGAHQAHLA	GKMALAKAPS	VASMDKSSCC	IVATSTQGSV	LPAWSAPREA
301	PDGGLFAVRR	HLWGSHGNSS	FPEFFHNMDY	FKYHNMRPPF	TYATLIRWAI
351	LEAPERQRTL	NEIYHWFTRM	FAYFRNHPAT	WKNAIRHNLS	LHKCFVRVES
401	EKGAVWTVDE	FEFRKKRSQR	PNKCSNPCP*		

Figure 2

# HUMAN FKHF cDNA Sequence

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1  GCACACACTC ATCGAAAABA ATTTGGATTA TTAGAAGAGA GAGGTCTGCC
51  GCTTCCACAC CGTACAGCGT GGTTCCTT CTCGGTATAA AAGCAAAGTT
101 GTTTTGTGATA CGTGACAGTT TCCCACAAGC CAGGCTGATC CTTTCTGTG
151 AGTCCACTTC ACCAAGCCTG CCCTTGGACA AGGACCCGAT GCCCAACCCC
201 AGGCCTGGCA AGCCCTCGGC CCCTTCCTTG GCCCTTGGCC CATCCCCAGG
251 AGCCTCGCCC AGCTGGAGGG CTGCACCCAA AGCCTCAGAC CTGCTGGGGG
301 CCCGGGGCCC AGGGGGAACC TTCCAGGGCC GAGATCTTCG AGGCGGGGCC
351 CATGCCTCCT CTTCTTCCTT GAACCCCATG CCACCATCGC AGCTGCAGCT
401 GCCCACACTG CCCCTAGTCA TGGTGGCACC CTCCGGGGCA CGGCTGGGCC
451 CCTTGCCCCA CTTACAGGCA CTCCTCCAGG ACAGGCCACA TTTCATGCAC
501 CAGCTCTCAA CGGTGGATGC CCACGCCCGG ACCCCTGTGC TGCAGGTGCA
551 CCCCTGGAG AGCCCAGCCA TGATCAGCCT CACACCACCC ACCACCGCCA
601 CTGGGGTCTT CTCCCTCAAG GCGCGGCCTG GCCTCCCACC TGGGATCAAC
651 GTGGCCAGCC TGAATGGGT GTCCAGGGAG CCGGCACTGC TCTGCACCTT
701 CCCAAATCCC AGTGCACCCA GGAAGGACAG CACCCTTTCG GCTGTGCCCC
751 AGAGCTCCTA CCCACTGCTG GCAAATGGTG TCTGCAAGTG GCCCGGATGT
801 GAGAAGGTCT TCGAAGAGCC AGAGGACTTC CTCAAGCACT GCCAGGCGGA
851 CCATCTTCTG GATGAGAAGG GCAGGGCACA ATGTCTCCTC CAGAGAGAGA
901 TGGTACAGTC TCTGGAGCAG CAGCTGGTGC TGGAGAAGGA GAAGCTGAGT
951 GCCATGCAGG CCCACCTGGC TGGGAAAATG GCACGTACCA AGGCTTCATC
1001 TGTGGCATCA TCCGACAAGG GTCCTGCTG CATCGTAGCT CTCTGCAGCC
1051 AAGGCCCTGT CGTCCCAGCC TGGTCTGGCC CCCGGGAGGC CCCTGCAGC
1101 CTGTTTGCTG TCCGGAGGCA CCGTGGGGT AGCCATGGAA ACAGCACATT
1151 CCCAGAGTTC CTCCACAACA TGGACTACTT CAAGTTCCAC AACATGCGAC
1201 CCCCTTTCAC CTACGCCACG CTCATCCGCT GGGCCATCCT GGAGGCTCCA
1251 GAGAAGCAGC GGACACTCAA TGAGATCTAC CACTGGTTCA CACGCATGTT
1301 TGCCTTCTTC AGAAACCATC CTGCCACCTG GAAGAACGCC ATCCGCCACA
1351 ACCTGAGTCT GCACAAGTGC TTTGTGCGGG TGGAGAGCGA GAAGGGGGCT
1401 GTGTGGACCG TGGATGAGCT GGAGTTCCGC AAGAAACGGA GCCAGAGGCC
1451 CAGCAGGTGT TCCAACCCTA CACCTGGCCC CTGACCTCAA GATCAAGGAA
1501 AGGAGGATGG ACGAACAGGG SCCAAACTGG TGGGAGGCAG AGGTGGTGGG
1551 GGCAGGGATG ATAGGCCCTG GATGTGCCCA CAGGGACCAA GAAGTGAGGT
1601 TTCCACTGTC TTGCCTGCCA GGGCCCCTGT TCCCCCGCTG GCAGCCACCC
1651 CCTCCCCCAT CATATCCTTT GCCCAAGGC TGCTCAGAGG GGCCCCGGTC
1701 CTGGCCCCAG CCCCCACCTC CGCCCCAGAC ACACCCCCCA GTCGAGCCCT
1751 GCAGCCAAAC AGAGCCTTCA CAACCAGCCA CACAGAGCCT GCCTCAGCTG
1801 CTCGCACAGA TTACTTCAGG GCTGGAAAAG TCACACAGAC ACACAAAATG
1851 TCACAATCCT GTCCCTCAC

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Figure 3

## HUMAN FKBP PROTEIN SEQUENCE

1 MPNPRPGKPS APSLALGPSP GASPSWRAAP KASDLLGARG PGGTFQGRDL  
51 RGGAHASSSS LNPMPPSQLQ LPTLPLVMVA PSGARLGPLP HLQALLQDRP  
101 HFMHQLSTVD AHARTPVLQV HPLESPAMIS LTPPTTATGV FSLKARGLP  
151 PGINVASLEW VSREPALLCT FPNPSAPRKD STLSAVPQSS YPLLANGVCK  
201 WPGCEKVFEED PEDFLKHCQA DHLLDEKGRA QCLLQREMVO SLEQQLVLEK  
251 EKLSAMQAHLE AGKMALTKAS SVASSDKGSC CIVAAAGSQGP VVPAWSGPRE  
301 APDSLFAVRR HLWGSHGNST FPEFLHNMDY FKFNMRPPF TYATLIRWAI  
351 LEAPEKQRTL NEIYHWFTRM FAFFRNHPAT WKNAIRHNLS LHKCFVRVES  
401 EKGAVWTVDE LEFRKKRSQR PSRCSNPTPG P\*

Figure 4

# Vector for generation of FKH<sup>sf</sup> Transgenic mice

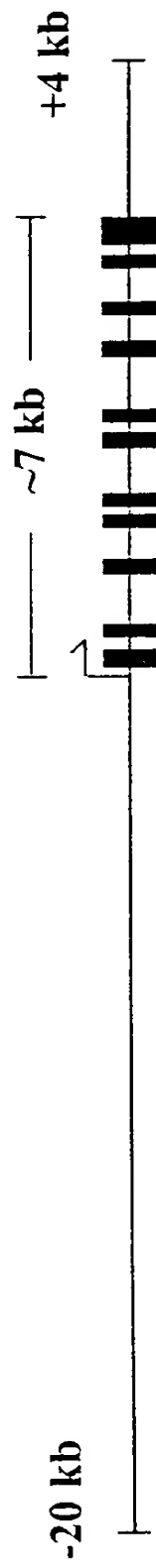


Figure 5

# FKHsf Transgene corrects the defect in scurfy animals

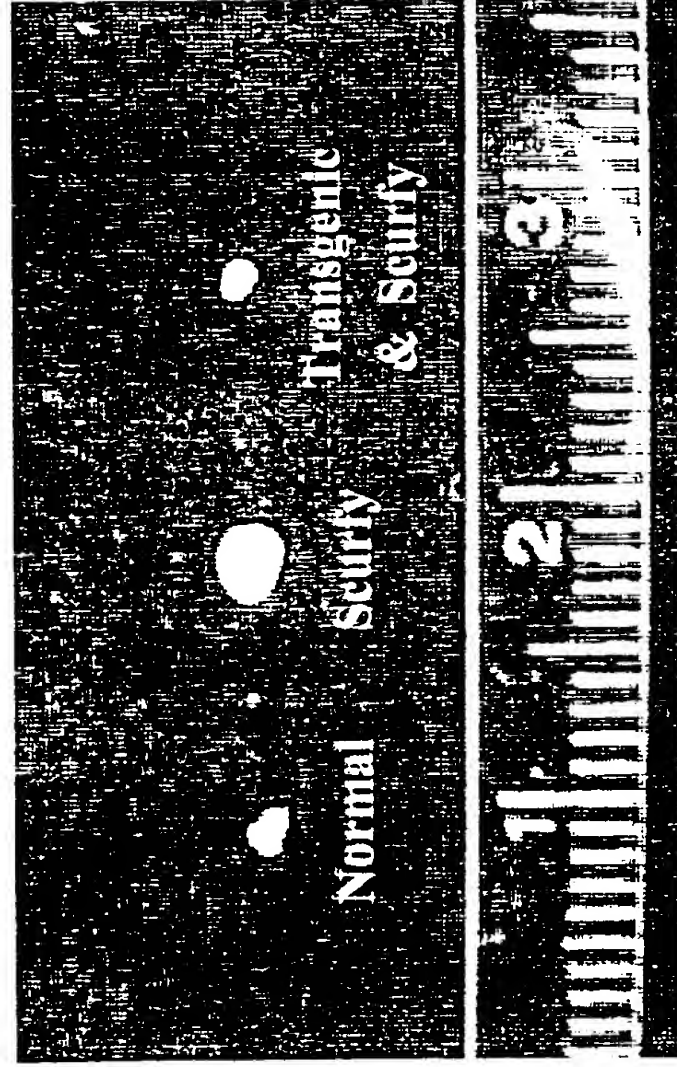


Figure 6

**FKHsf tg mice have reduce lymph node cells  
compared to normal cells**

	Mouse genotype		
	Normal	Scurfy	Transgenic
Cell number			
Cells / LN	0.92	1.97	0.29
Cells / Thymus	0.76	0.54	0.76

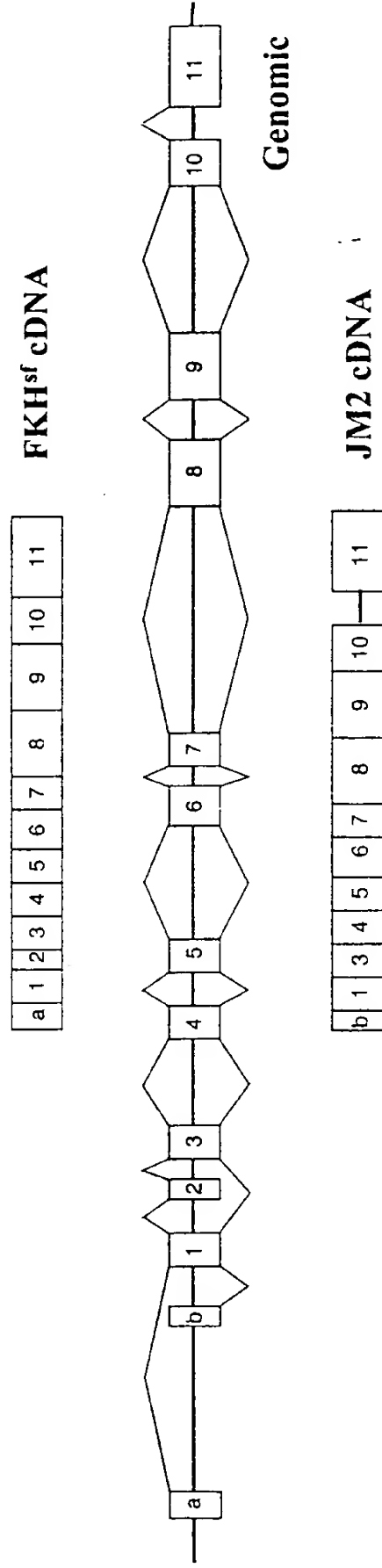
**Figure 7**

# FKHsf transgenic mice respond poorly to in vitro stimulation

Proliferation	Mouse genotype	
	Normal	Scurfy Transgenic
No stimulation	778	23488
Anti-CD3+Anti-CD28	22932	225981
		9106

Figure 8





**Comparison of FKH<sup>sf</sup> and JM2 cDNAs.** Exon/intron structure is shown (Genomic) as open rectangles (exons) joined by heavy horizontal lines (introns). Coding exons are numbered 1-11 as determined by sequence analysis of FKH<sup>sf</sup> cDNA; non-coding 5' exons are labelled *a* and *b*. The FKH<sup>sf</sup>-specific and JM2-specific splicing patterns and resulting cDNAs are indicated above and below the genomic structure, respectively.

**Figure 9**

<u>Human FKHS<sup>f</sup></u>			
<i>N-terminal</i>	<i>ZNF</i>	<i>Mid</i>	<i>Forkhead</i>
83.4%	95.8%	82.8%	96.4%
<u>Mouse Fkhs<sup>f</sup></u>			

Human and mouse FKHS<sup>f</sup> proteins are highly conserved.

**Figure 10**